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RE: TWO PUBLICATIONS ADDRESSING FORMALDEHYDE EPIDEMIOLOGY THAT ARE KEY TO IRIS EVALUATION

Date July 24, 2017

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Dear Dr. Yamada:

Congratulations on your recent appointment as EPA Deputy Assistant Administrator for Research & Development (ORD).

As a research epidemiologist for 30 years, I have conducted and published epidemiological studies and reviews relevant to EPA's Integrated Risk Information System (IRIS) evaluations of various chemicals. I am aware that the **IRIS Toxicological Review of Formaldehyde (Inhalation) (External Review Draft 2010)** is currently being revised and I wish to draw to your attention two publications that I have co-authored, both central to the evaluation of formaldehyde as a possible human leukemogen. Both also directly challenge the validity of the two predecessor studies conducted or funded by the US National Cancer Institute (NCI) and heavily relied upon in evaluations of formaldehyde carcinogenicity conducted by both IRIS and the International Agency for Research on Cancer (IARC).

In May, I authored a paper, "Does occupational exposure to formaldehyde cause hematotoxicity and leukemia-specific chromosome changes in cultured myeloid progenitor cells?," presenting a detailed analysis of data obtained from NCI examining potential correlations between formaldehyde exposure and prevalence of aneuploidy and other blood measures.[1] No associations were seen across levels of measured formaldehyde exposure for any outcome. On May 2, 2017, I sent a copy of this study to Drs. Bahadori (NCEA) and Thayer (IRIS) and other IRIS staff. Receipt was acknowledged but I have received no further requests to discuss findings or address any questions the Agency might have. I also have written a letter to the editor of the journal that published the original analyses, highlighting

[1] Mundt, K. A., Gallagher, A. E., Dell, L. D., Natelson, E. A., Boffetta, P., & Gentry, P. R. (2017). Does occupational exposure to formaldehyde cause hematotoxicity and leukemia-specific chromosome changes in cultured myeloid progenitor cells? Critical Reviews in Toxicology, 1-11, Received 28 Oct 2016, Accepted 27 Feb 2017, Published online: 02 May 2017.

some methodological problems including failure to use the actual exposure data collected and subsequent incomplete data analysis. This letter has been accepted for publication, and will appear soon in **Cancer Epidemiology Biomarkers and Prevention**, with a response from the original investigators. I attach copies of both the published article and the letter.

In 2015, I co-authored a paper, similarly based on fuller statistical analyses of data obtained as part of another influential NCI epidemiology study: "Formaldehyde exposure and mortality risks from acute myeloid leukemia and other lymphohematopoietic malignancies in the US National Cancer Institute cohort study of workers in Formaldehyde Industries" (Checkoway, et al. 2015).[2] This paper, which just received the 2017 Adolf G. Kammer Merit in Authorship Award from the American College of Occupational and Environmental Medicine, presents more thorough analyses of data previously evaluated by NCI investigators. The original publication reported an association between "peak" formaldehyde exposure and myeloid leukemias combined; however, our detailed analysis demonstrated that there was no such relationship between any formaldehyde exposure metric (including "peak") and acute myeloid leukemia – the type of myeloid leukemia most plausibly related to environmental exposures. I sent a copy of the published paper to Dr. Vincent Cogliano on July 15, 2015, receipt of which was acknowledged. I attach a copy here as well.

In addition to these, I have co-authored the following publications relevant to the IRIS formaldehyde evaluation:

- Van Landingham, C., Mundt, K. A., Allen, B. C., and Gentry, P. R. (2016). The need for transparency and reproducibility in documenting values for regulatory decision making and evaluating causality: The example of formaldehyde. **Regulatory Toxicology and Pharmacology**, 81, 512-521.
- Checkoway, H., Boffetta, P., Mundt, D., and Mundt, K. (2012). Critical review and synthesis of the epidemiologic evidence on formaldehyde exposure and risk of leukemia and other lymphohematopoietic malignancies." **Cancer Causes & Control** 23, no. 11: 1747-1766.

I would welcome the opportunity to meet with you and your staff to discuss these studies and other research on formaldehyde carcinogenicity that might be informative, and supportive of a scientifically robust IRIS assessment. I can be reached by phone at 1 (413) 835-4360 or email: kmundt@ramboll.com
Thank you, and I look forward to your reply.

Yours sincerely

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[2] Checkoway, H., Dell, L.D., Boffetta, P., Gallagher, A.E., Crawford, L., Lees, P.S., and Mundt, K.A. (2015). Formaldehyde exposure and mortality risks from acute myeloid leukemia and other Lymphohematopoietic Malignancies in the US National Cancer Institute cohort study of workers in Formaldehyde Industries. Journal of Occupational and Environmental Medicine, 57(7), 785-794.

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REVIEW ARTICLE

Does occupational exposure to formaldehyde cause hematotoxicity and leukemia-specific chromosome changes in cultured myeloid progenitor cells?

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ABSTRACT

Several cross-sectional studies of a single population of workers exposed to formaldehyde at one of two factories using or producing formaldehyde–melamine resins in China have concluded that formaldehyde exposure induces damage to hematopoietic cells that originate in the bone marrow. Moreover, the investigators interpret observed differences between groups as evidence that formaldehyde induces myeloid leukemias, although the mechanisms for inducing these diseases are not obvious and recently published scientific findings do not support causation. Our objective was to evaluate hematological parameters and aneuploidy in relation to quantitative exposure measures of formaldehyde. We obtained the study data for the original study (Zhang et al. 2010) and performed linear regression analyses. Results showed that differences in white blood cell, granulocyte, platelet, and red blood cell counts are not exposure dependent. Among formaldehyde-exposed workers, no association was observed between individual average formaldehyde exposure estimates and frequency of aneuploidy, suggested by the original study authors to be indicators of myeloid leukemia risk.

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Formaldehyde; hematopoietic cells; leukemia; myeloid leukemia; acute myeloid leukemia

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Introduction

The International Agency for Research on Cancer (IARC) evaluated the carcinogenicity of formaldehyde in October, 2009, and according to Monograph 100F, "on balance, the Working Group concluded that the epidemiologic evidence shows that occupational exposure to formaldehyde causes leukemia" (IARC 2012). However, Monograph 100F noted that this determination was not unanimous, and a small majority viewed the evidence as sufficient of carcinogenicity while a minority viewed the evidence as limited. Monograph 100F further stated:

> Particularly relevant to the discussions regarding sufficient evidence was a recent study accepted for publication which, for the first time, reported aneuploidy in blood of exposed workers characteristic of myeloid leukaemia and myelodysplastic syndromes, with supporting information suggesting a decrease in the major circulating blood-cell types and in circulating haematological precursor cells. The authors and Working Group felt that this study needed to be replicated.

The specific study referred to here was "Occupational Exposure to Formaldehyde, Hematotoxicity, and Leukemia-Specific Chromosome Changes in Cultured Myeloid Progenitor Cells" by Dr. Luoping Zhang and 33 coauthors, accepted for publication one week before the IARC Working Group convened on 20 October 2009, and officially published online on 7 January 2010 (Zhang et al. 2010).

In 2010, the US Environmental Protection Agency (EPA) Integrated Risk Information System (IRIS) issued a Draft Toxicological Review of Formaldehyde (referred to hereafter as the IRIS Draft). Although the IRIS Draft has not yet been finalized, it stated that Zhang et al. (2010) provided "the best evidence for bone marrow toxicity, where they report not only a

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reduction in white blood cell counts, but reductions in cell counts of all the blood cells, as well as increased mean cell volume" (EPA 2010). The IRIS Draft also noted that

> ... the results of Zhang et al. (2010) need to be extended (analysis for additional chromosomal aberrations) and repeated. Although further evidence is needed to better understand the hypothesized mechanisms for formaldehyde-induced effects on hematopoietic stem cells, the observed hematologic effects in humans cannot be set aside. Therefore, however unlikely, the current data support the biological plausibility of formaldehyde effects on the hematopoietic system (EPA 2010).

This highly influential study – which has not been replicated to date – was a cross-sectional statistical evaluation comparing blood parameters considered by the authors to be indicators of hematotoxicity and chromosomal changes in myeloid progenitor cells, specifically monosomy 7 and trisomy 8. However, no actual changes in any of the parameters were measured; rather, differences between groups were observed. These parameters have been found to be more common among individuals with acute myeloid leukemia (AML) but are not associated with chronic myeloid leukemia (CML). Comparisons were made between 43 workers exposed to formaldehyde and 51 unexposed controls, based strictly on exposure represented as a dichotomous variable (exposed versus unexposed) and not considering individual level exposure data. The exposed group included workers if they had formaldehyde exposure levels "of about 1–2 ppm [parts per million] on most days during the initial screening" and had worked in the same job for the previous three months (Zhang et al. 2010). Of the 43 exposed subjects included in the study, 41 (95%) had worked for at least one year in either of two factories that produced or used formaldehyde-melamine resins. The formaldehyde exposure was characterized by the authors as "relatively high levels of formaldehyde (mostly between 0.6 and 2.5 ppm)" (Zhang et al. 2010). The 51 unexposed workers were selected from three separate workplaces (reported by Bassig et al. 2016 to be two clothes manufacturing factories and one food production factory) in the same region, with no occupational formaldehyde exposure (verified via workplace sampling), and frequency matched on age (± 5 years) and sex (Zhang et al. 2010).

Blood samples from these two groups of exposed and unexposed workers have been included in additional evaluations of aneuploidy and structural chromosome aberrations (SCAs) of all 24 chromosomes (Lan et al. 2015), and in comparisons of hematotoxicity, monosomy 7 in myeloid progenitor cells (MPCs) and B-cell activation biomarkers across groups exposed to benzene, formaldehyde, and trichloroethylene (Bassig et al. 2016). In all three publications, differences in blood parameters and genetic markers of the group of workers exposed to formaldehyde are compared with those of the unexposed group, i.e. ecologically. Specifically, in the report relied upon by the IARC Working Group (Zhang et al. 2010), statistically significant differences were reported for several blood parameters, as well as increased aneuploidies (monosomy 7 and trisomy 8) in myeloid progenitor cells in comparing results from formaldehyde exposed workers and unexposed controls (Zhang et al. 2010). The analyses were based on the OctoChrome FISH protocol developed and marketed by some of the same investigators (Zhang et al. 2005). Based on these findings, Zhang et al. (2010) proposed that formaldehyde exposure may have damaged hematopoietic cells, and therefore, provides support for the hypothesis that formaldehyde causes myeloid leukemia, and presumably AML specifically.

However, in none of these reports (i.e. Zhang et al. 2010; Lan et al. 2015; Bassig et al. 2016) are the individual formaldehyde exposure measures (or mean of these) among the "exposed" workers evaluated for their relationship, if any, with the reported outcome measures. Nor were the individual formaldehyde exposure estimates divided into ranges of exposure for analysis with the blood and aneuploidy outcomes as was done with benzene and trichloroethylene exposure estimates in the study by Bassig et al. (2016). Individual formaldehyde exposure measurements clearly were available, as each of the reports describes the sampling methods used, for example: "Personal FA exposure was monitored with SKC UMEx 100 passive samplers, which were worn by workers in the exposed factories for a full work shift for about three workdays over a 3-week period" (Bassig et al. 2016). Averages of the actual exposure measurements were used to estimate individual formaldehyde estimates for each of these workers. However, the authors ultimately treat all concentrations of formaldehyde exposure among the "exposed" workers as the same, despite a fourfold 10th–90th percentile exposure range (0.6–2.5 ppm), which the authors claimed was insufficient to differentiate risks by actual exposure levels: In a subsequent publication of the same underlying data, Lan et al. (2015) reported "The study was designed to evaluate mechanistically relevant biomarkers in workers exposed to relatively high levels of FA, and as a consequence there was a relatively narrow range of exposure that precluded assessment of exposure-response relationships." However, the authors of these reports fail to consider that unmeasured differences between the exposed and unexposed groups – other than their formaldehyde exposure – likely contributed to the differences observed at the group level, and that some association, if present, would be seen across this more than fourfold range of individual exposure estimates and some of the blood and aneuploidy measures.

Gentry et al. (2013) obtained most of the Zhang et al. (2010) data from the National Cancer Institute (NCI), through a Freedom of Information Act (FOIA) request. The individual formaldehyde exposure measurement data, however, were not provided. In brief, the Gentry et al. (2013) re-analysis did not substantiate the original study claim that monosomy 7 and trisomy 8 arose in vivo in hematopoietic stem cells from humans exposed to formaldehyde. They noted that based on the kinetics of CFU-GM colony formation, the reported aneuploidies observed could not have arisen in vivo, but most likely occurred in vitro during cell culture (Gentry et al. 2013). This has been reiterated by Albertini and Kaden (2017). Gentry et al. (2013) also detected and reported significant methodological limitations, including the discovery that the authors did not follow their own protocol, which specified the number of cells to be scored from each study participant. This information was not included in the Zhang et al. (2010) publication and was only determined through the acquisition

of the raw data from the study through the FOIA request. In fact, cultures from only one and three exposed workers respectively met the criterion specified in the Zhang study protocol (Zhang et al. 2010) that a minimum of 150 cells would be scored for both the monosomy 7 and trisomy 8 evaluations (Gentry et al. 2013). Gentry et al. (2013) concluded that their reanalyses "raise sufficient questions that limit the use of Zhang et al. (2010) to support the hypothesis that formaldehyde exposure is causally related to leukemia or lymphoid malignancies" (Gentry et al. 2013). They also recommended that exposure–response analyses would be helpful in verifying that occupational exposure to formaldehyde "damages hematopoietic stem or early progenitor cells in the bone marrow and/or peripheral blood" as reported by Zhang et al. (2010) (Gentry et al. 2013).

In 2014, we requested the individual exposure measurement data for each of the participants in the Zhang et al. (2010) study. In 2016, our request was in part granted and the mean formaldehyde estimate for each exposed worker (but not the individual exposure measurement values) was provided via a Technology Transfer Agreement (TTA) with the NCI. In this report, we extend the Gentry et al. (2013) reanalysis using the additional data provided to perform exposure-response analysis.

Methods

Demographic and exposure characteristics of study subjects as reported by Zhang et al. (2010) were replicated. As reported by Zhang et al. (2010), formaldehyde exposure among the exposed subjects was estimated based on formaldehyde monitoring performed using diffusion samplers (limit of detection ¼ 0.012 ppm) "for a full shift (>240 min) on ┐3 working days over a 3-week period." For the exposed group, Zhang et al. (2010) reported the median of the summary 8-hour time-weighted average (8-h TWA) measurement and the 10th and 90th percentiles of the summary measurements. Using the summary TWA measurement for each exposed worker, we categorized participants into "lower" and "higher" exposure groups based on the overall median exposure level (1.3 ppm).

Zhang et al. (2010) reported that the assigned exposure values in controls were based on the 8-h TWA level in their respective control factories based on measurements performed for a subgroup of workers. Study subjects with non-detectable formaldehyde exposure were assigned a value of the limit of detection divided by the square root of two. Based on this information, seven non-exposed workers had been assigned 0.0085 ppm by Zhang et al. (2010), consistent with a limit of detection of 0.012 ppm. In addition, 14 unexposed workers had been assigned an intensity of 0.0146 ppm and 27 unexposed workers had been assigned an intensity of 0.0262 ppm as an 8-h TWA. Estimated exposures in the exposed workers were 8-h TWAs based on the arithmetic mean of the individual's exposure measurements (which were not provided by NCI) and ranged from 0.318 to 5.61 ppm among exposed workers (Figure 1).

As described (Zhang et al. 2010), peripheral blood samples were collected from study subjects in the workplace and from the formaldehyde exposed workers after they had been monitored at least twice. Complete blood counts with differential and lymphocyte subsets were measured for each study subject. Cells defined by the authors as hematological progenitor cells (peripheral blood mononuclear cells) were cultured using the colony forming unit-granulocyte/macrophage (CFU-GM) assay. Metaphases from CFU-GM cells were prepared after 14 days (d) of culture. Two types of chromosomal markers that the authors described as "among the most frequent cytogenetic changes observed in myeloid leukemia and myelodysplastic syndromes," specifically, monosomy 7 and trisomy 8, were examined in CFU-GM cells using fluorescence in situ hybridization (FISH) staining of metaphase spreads (Zhang et al. 2005). Each metaphase spread was examined microscopically for 10 workers chosen from those with the highest formaldehyde exposure and 12 unexposed controls frequency matched to the exposed workers by age and sex (Zhang et al. 2010). Frequency matching allowed for the control of age and sex in the analysis.

In the present analysis, exposure values for each worker were linked with the eight blood count parameters

Table 1. Association between formaldehyde exposure and the blood parameters.

Blood parameter	Unadjusted Exp(b[a])	95% CI	p value	Adjusted Exp(b[a])[b]	95% CI	p[c] value
WBC						
Unexposed	Reference			Reference		
< 1.3 ppm	0.85[d]	0.76–0.96		0.87	0.78–0.97	
└ 1.3 ppm	0.86	0.76–0.97	.992	0.85	0.76–0.96	.943
Lymphocytes						
Unexposed	Reference			Reference		
< 1.3 ppm	0.83	0.73–0.95		0.85	0.75–0.96	
└ 1.3 ppm	0.80	0.70–0.92	.890	0.79	0.69–0.90	.660
Monocytes						
Unexposed	Reference			Reference		
< 1.3 ppm	0.86	0.72–1.04		0.90	0.77–1.06	
└ 1.3 ppm	0.92	0.76–1.11	.856	0.89	0.75–1.04	.973
Granulocytes						
Unexposed	Reference			Reference		
< 1.3 ppm	0.86	0.74–1.00		0.87	0.75–1.01	
└ 1.3 ppm	0.89	0.76–1.04	.931	0.88	0.75–1.03	.997
RBC						
Unexposed	Reference			Reference		
< 1.3 ppm	0.94	0.89–0.99		0.94	0.91–0.98	
└ 1.3 ppm	0.94	0.89–1.00	.999	0.94	0.90–0.98	.947
Hemoglobin						
Unexposed	Reference			Reference		
< 1.3 ppm	0.97	0.92–1.02		0.98	0.94–1.01	
└ 1.3 ppm	1.00	0.94–1.05	.667	0.99	0.95–1.03	.818
Platelets						
Unexposed	Reference			Reference		
< 1.3 ppm	0.85	0.76–0.96		0.85	0.75–0.96	
└ 1.3 ppm	0.91	0.80–1.03	.695	0.91	0.80–1.03	.674
MCV						
Unexposed	Reference			Reference		
< 1.3 ppm	1.03	0.99–1.07		1.03	0.99–1.08	
└ 1.3 ppm	1.06	1.02–1.11	.379	1.06	1.02–1.11	.550

[a]Regression coefficient between log-transformed blood parameter and formaldehyde.
[b]Adjusted for combined sex/smoking variable.
[c]p values for pairwise comparison between < 1.3 ppm and └ 1.3 ppm categories.
[d]Bolded results are statistically significantly different from the reference group.

Table 2. Monosomy of chromosome 7 (| 7) and trisomy of chromosome 8 (þ8) in peripheral blood cells scored by Zhang et al. (2010) – updated table from Gentry et al. (2013) and sorted by average intensity of formaldehyde.

FA ppm	Smoking status	Total cells scored	Abnormal metaphases | 7	Frequency | 7 (%)	Total cells scored	Abnormal metaphases þ8	Frequency þ8 (%)
Produced or used melamine formaldehyde resins (n¼10)							
5.61	No	109	4	3.7	139	0	0.0
2.68	Yes	76	9	11.8	149	1	0.7
2.60	Yes	123	20	16.3	173	4	2.3
2.32	No	39	6	15.4	61	2	3.3
2.29	Yes	274	11	4.0	180	4	2.2
2.00	Yes	132	15	11.4	192	2	1.0
1.99	No	50	10	20.0	78	2	2.6
1.94	No	95	3	3.2	108	0	0.0
1.38	No	101	4	4.0	53	0	0.0
1.38	No	61	13	1.38	33	0	0.0
Worked in control factories (n¼12)							
0.03	No	272	10	3.7	226	2	0.9
0.03	Yes	260	10	3.8	215	2	0.9
0.03	No	163	8	4.9	91	0	0.0
0.03	Yes	140	6	4.3	69	0	0.0
0.03	No	78	2	2.6	83	0	0.0
0.03	No	71	1	1.4	37	0	0.0
0.03	Yes	20	2	10.0	25	0	0.0
0.03	Yes	18	1	5.6	21	0	0.0
0.01	Yes	288	19	6.6	197	2	1.0
0.01	No	70	9	12.9	94	1	1.1
0.01	No	49	4	8.2	67	0	0.0
0.01	No	24	0	0.0	22	0	0.0

Shaded cells represent samples following reported methodology (analyzed └ 150 cells).

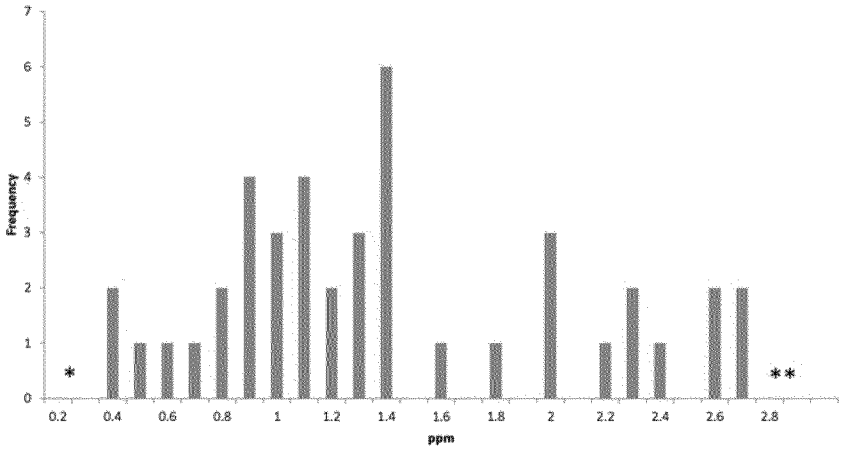

Figure 1. Distribution of inhalable formaldehyde measurements in workers (n¼43) from exposed factories. ┘Formaldehyde exposure for the 51 workers from unexposed workplaces is not included. ┘┘The maximum outlier of 5.6 ppm has not been included in this figure.

(discussed below), and, where applicable, the aneuploidy results. Characteristics of the study participants, including means, standard deviations, medians, and ranges for blood parameters were described and stratified by exposure status (exposed/not exposed to formaldehyde). This allowed further insight into variability of blood parameters among the study subjects (formaldehyde-exposed and controls) as well as identified potential confounders, when stratified by exposure status (exposed/not exposed to formaldehyde). In addition, median values for blood parameters and ranges were compared between exposed and unexposed groups, and individual values for blood parameters were compared to reference intervals for the Chinese population (Wu et al. 2015) to identify individual values that fall outside normal ranges. All analyses were conducted using SAS 9.3 (SAS Inc., Cary, NC).

Indicators of hematotoxicity

Each of the blood count parameters, specifically, white blood cell (WBC) count and its component lymphocytes, monocytes, and granulocytes; red blood cell (RBC) count and its component hemoglobin and platelets; and mean corpuscular volume (MCV), was examined as the primary outcome variables of interest. Results as reported in Zhang et al. (2010), i.e. comparing exposed and unexposed groups, were verified. Additional stratified analyses were conducted among the exposed group only using the median cut-point, as well as linear regression analyses using the individual exposure estimates and relevant covariates and the natural logarithm of the blood count data. Age, body mass index (BMI), sex, current smoking, current alcohol consumption, recent respiratory infections, recent use of Chinese medicine, and recent use of Western medicine all

were examined as possible covariates. Thalassemia trait was also considered and was defined as those blood samples with MCV values of less than 70 femtoliters (fl), as values below this level are believed to provide a possible indication of the thalassemia trait. As reported by Gentry et al. (2013), thalassemia, an inherited blood disease, decreases MCV and increases RBC counts, so thalassemia is a possible confounder of the association between formaldehyde exposure and RBC and MCV. To address this, we ran sensitivity analyses after excluding five workers with MCV levels of less than 70 fl.

The variables included in the adjusted models were guided by the descriptive analysis. The unexposed and exposed workers were similar in terms of age and sex, as would be expected as a result of the frequency matching that was used in selecting unexposed controls; however, only 14% of the study participants were women. Because there were no women who reported current smoking, we combined smoking and gender variables (into groups of male smokers, male non-smokers, and female non-smokers), allowing contrasts to be made by gender and smoking individually and jointly.

Aneuploidy

Zhang et al. (2010) reportedly analyzed monosomy 7 and trisomy 8 based on the percentage observed in each sample, which was determined by dividing the number of aneuploidies observed for each subject by the number of cells counted in vitro. The strong case challenging the biological rationale of the CFU-GM analysis presented by Gentry et al. (2013), as well as the very small sample sizes reported, argue against performing additional statistical analyses for these outcomes by individual formaldehyde exposure estimates. Nevertheless, we provide descriptive and graphical results in relation to test result, reliability (based on actual counts versus 150 required by the protocol), smoking and formaldehyde exposure estimate.

Results

Indicators of hematotoxicity

Among women classified as non-smokers, no differences in any of the blood parameters were detected between the exposed workers and unexposed workers (Supplemental Table 1). There were no women who smoked. Among men classified as non-smokers, WBC, lymphocyte, and RBC were higher in the unexposed workers compared with the exposed workers. Among male smokers, lymphocytes were higher and MCV was lower in the unexposed compared with the exposed. Mean blood parameters for exposed and unexposed workers were summarized according to gender and smoking status (Supplemental Table 1). As expected, smokers had higher WBC counts than non-smokers, irrespective of exposure status, although male non-smokers appeared to have higher WBC counts than female non-smokers for both exposed and unexposed workers. Among unexposed workers, for example, white blood cell counts were 5064.3 per ll in women (all non-smokers), compared with 6093.3 per ll in men who were non-smokers and 6796.5 per ll in men who were smokers. Statistically significant differences in means between exposed and unexposed workers were observed for WBC counts and RBC counts in male non-smokers, but not male smokers. Among both male smokers and male non-smokers, statistically significant differences in means between exposed workers and unexposed workers were seen for lymphocyte counts.

Although trend tests were statistically significant in untransformed models of WBC, RBC, and lymphocyte counts, exposure-dependent differences in these parameters were not apparent when formaldehyde exposure was categorized according to median concentration in the exposed workers, and adjusting for smoking and sex (Figure 2).

In log-transformed models of blood parameters adjusted for sex and current smoking, WBC, RBC, and lymphocyte counts were lower in the formaldehyde exposed workers compared with the unexposed workers, but the differences were of similar magnitude in both exposure categories (<1.3 ppm, ≥1.3 ppm) (Table 1). Specifically, compared with the unexposed, WBCs were 13–15% lower, lymphocyte counts were 15–21% lower, and RBCs were 6% lower. Additionally, MCV was 6% higher in the ≥1.3 ppm formaldehyde exposure category only compared with the unexposed, and platelet counts were 15% lower in the <1.3 ppm formaldehyde exposure category only compared with the unexposed. Results in unadjusted linear regression models were similar (Table 1).

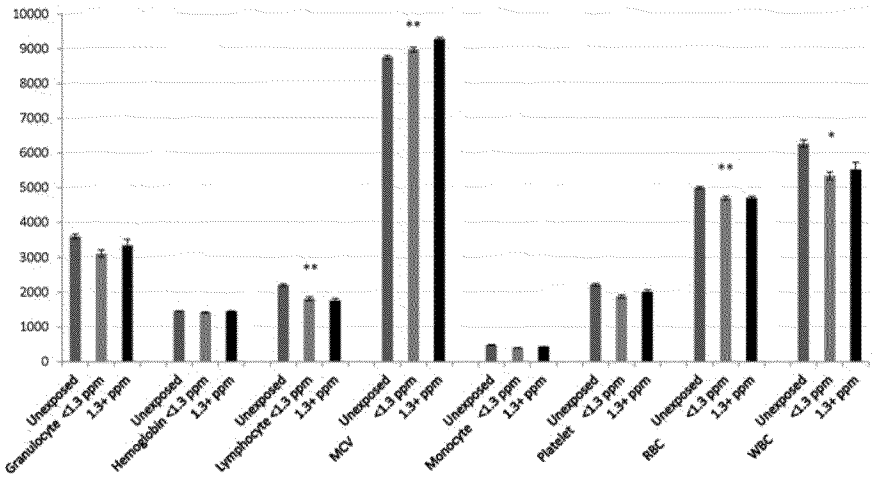

Figure 2. Cell counts per ll blood by formaldehyde exposure. Untransformed means/cells ml and standard errors. All models adjusted for sex and current smoking. Hemoglobin and MCV values are reported in g/dL and fl, respectively, and have been multiplied by 100 to make them visible. RBC values were multiplied by 1000. Platelets were multiplied by 100. *Ptrend <0.05; **Ptrend <0.01.

Sex and smoking were associated with some of the blood parameters in the adjusted models. Statistically significant differences in monocyte, RBC, and hemoglobin counts were detected for the combined variable of current smoking status and sex. Compared with male non-smokers, monocyte counts were 24% higher in male smokers (p¼.0002) and 33% lower in female non-smokers (p¼.003). RBC counts were 20% lower in female non-smokers compared with male non-smokers ($p < .0001$) and 4% lower in male smokers compared with male non-smokers (p¼.031). Hemoglobin counts were 18% lower in female non-smokers compared with male non-smokers ($p < .0001$); no differences in hemoglobin were detected between male smokers and male non-smokers.

In the sensitivity analysis, removing five subjects with presumed thalassemia trait did not substantially modify the results for MCV or RBC. As noted previously, thalassemia, an inherited autosomal recessive blood disease common in Asian populations, is associated with a decrease in MCV and an increase in RBC counts. In addition, removing these individuals from consideration did not change the overall conclusions for any of the other blood parameters measured (data not shown).

We also generated models of blood parameters that included the exposed workers only with formaldehyde modeled as a continuous variable (Supplemental Table 2). No significant differences in any of the blood parameters were seen with each one ppm increase in formaldehyde exposure, adjusting for sex and smoking.

Finally, we compared means of the blood parameters for exposed and unexposed workers with the reference intervals for healthy Chinese adult men and women (Wu et al. 2015). We extended the comparison by Gentry et al. (2013) by identifying the number of workers in each category that fell outside of the reference interval (Supplemental Table 3). Although the sample size was small, which limited formal statistical analysis, few workers had blood count values that fell outside of the reference ranges. For WBC counts and its components (lymphocytes, monocytes, and granulocytes), none of the women fell outside of the normal ranges. Among men, two exposed workers had low WBC counts while one exposed worker and one unexposed worker had high WBC counts. No exposed men had lymphocyte counts that fell outside of the normal ranges. Four exposed men had monocyte counts that were higher than normal values; however, 13 unexposed men had monocyte counts that were higher than normal values. Three exposed men had granulocyte counts that were lower than normal values and one exposed man had granulocyte counts that were higher than normal values.

Aneuploidy

Zhang et al. (2010) analyzed monosomy 7 and trisomy 8 in a subset of 10 "highly exposed" workers and 12 matched controls (Table 2). These data are plotted according to average intensity of formaldehyde for monosomy 7 and trisomy 8 (Figure 3). Few subjects had adequate numbers of CFU-GM progenitor cells analyzed to meet the study protocol criteria of evaluating >150 cells. The lack of compliance with the study protocol is critical, as the cutoff or background for FISH results is expected to be above zero and no cutoff was established for this analysis. The normal cutoff for an analysis of 200 cells can be as high as approximately 5%, depending on the number of false positives identified in the normal specimens (Wolff et al. 2007). Typically, in the clinical setting 200–400 cells are scored and cutoffs determined based on the false positives previously defined from normal specimens.

When considering the protocol established by Zhang et al. (2010), for monosomy 7, only a single exposed worker and four controls met the criterion of scoring 150 cells, while for trisomy 8, only three exposed workers and three controls met the criterion (Table 2). In addition, considering that the cutoff for these analyses would not be zero and assuming it could potentially be in the range of 2–5%, approximately half of the monosomy 7 findings could be below the cutoff, with the majority of trisomy 8 findings below the cutoff. Regardless of the number of cells considered, however, no pattern between formaldehyde exposure and the frequency of monosomy 7 was observed (Figure 3). Sensitivity analyses revealed that the frequency of monosomy 7 in workers with fewer than 80 cells scored is highly sensitive to small changes in the number of cells included. For example, the frequency of monosomy 7 in a subject with 78 scorable cells would change by more than 1% with the detection of one additional (or one fewer) abnormality (e.g. actual: 2/78 (2.6%) to 3/78 (3.8%) with one additional abnormality detected) and the uncertainty proportionately greater with fewer counts. This highlights the potential impact of results from subjects for which the appropriate number of cells (based on the criterion defined by Zhang et al. 2010) were not scored.

No pattern between formaldehyde exposure and trisomy 8 was observed (Figure 3). Of note, all the selected exposed workers who additionally met the research protocol were also smokers.

Discussion

The Zhang et al. (2010) study was highly influential in the evaluation of formaldehyde as a plausible human leukemogen, and as noted above, was specifically cited by IARC (2012) and in the draft EPA (2010) formaldehyde IRIS assessment as providing evidence to support plausible mechanisms by which formaldehyde exposure may cause leukemogenesis. This recognition came despite the fact that primary evaluations reported by Zhang et al. (2010) of aneuploidies and indicators of hematotoxicity were limited to fairly crude aggregation of workers from different industries into "exposed" and "unexposed" categories. However, the most serious problems underlying the study may not have been apparent to the evaluation committees, because the limitations regarding analyses of dichotomous formaldehyde exposure (exposed versus unexposed), as well as measurement of aneuploidy (whether the reported aneuploidies could have occurred during cell culture in vitro) were not reported by the original authors. The study investigators also failed to acknowledge that the differences seen between the exposed and unexposed groups could reflect other underlying differences between the employees at different study factories. Additional information about the two groups beyond the few available occupational

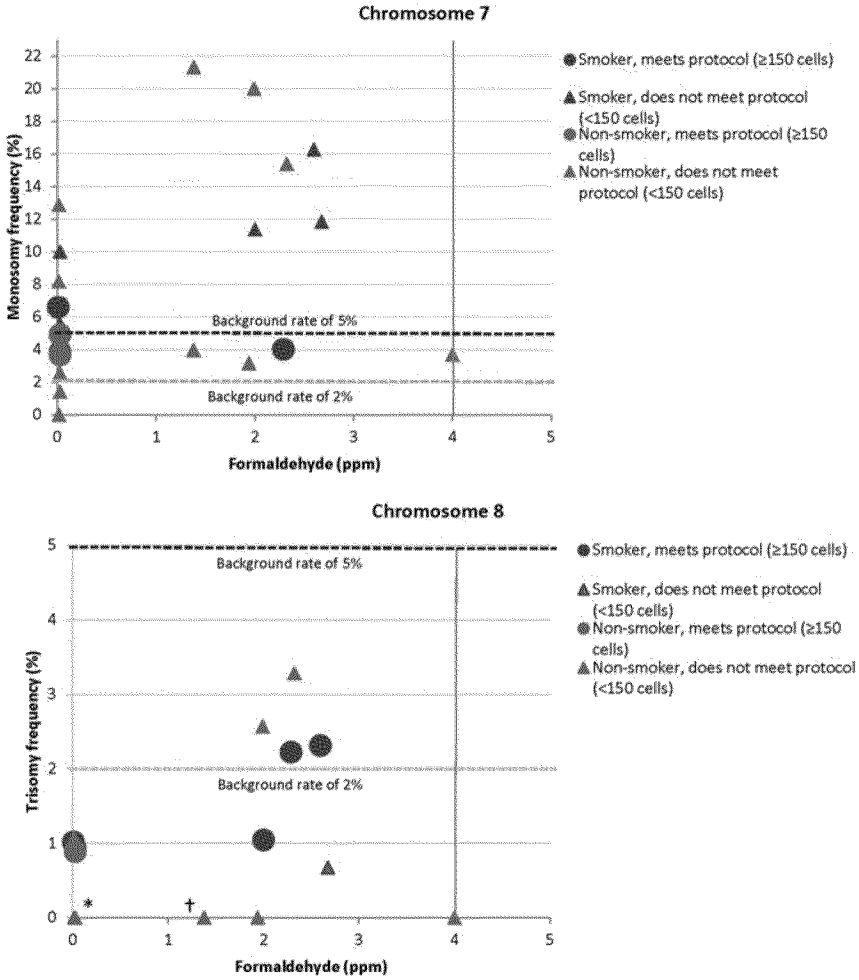

Figure 3. Individual average formaldehyde intensity for monosomy 7 and trisomy 8. ⌟ Represents 8 subjects. † Represents 2 subjects. Vertical line represents the maximum value of 5.6 ppm, plotted at 4 ppm to improve readability of the figure.

and individual characteristics would be needed in order to more fully evaluate these differences. Furthermore, the publication was not generally available for full review and evaluation prior to the IARC working group meeting. Zhang et al. (2010) was accepted for publication one week before the meeting, and published online two months after the meeting (Baan et al. 2009). Study data were not shared for re-evaluation until years after they were requested (Gentry et al. 2013). However, the EPA (2010) IRIS assessment for formaldehyde is still in draft form, and the results of the analyses presented here should help place the original interpretations of the reported study findings into the proper context.

One of the major criticisms of the Zhang et al. (2010) study noted by Gentry et al. (2013) was the decision not to present any results by estimated individual exposure level, which would have provided a fuller evaluation and much stronger evidence of an association, should one exist. Although Gentry et al. (2013) had obtained most of the Zhang et al. (2010) study data through a FOIA request, the raw exposure measurement data and the summary variable were withheld. This prevented fuller evaluation using individual exposure estimates. The individual mean exposure estimates, however, eventually were provided to us by NCI, and we have completed and reported here the results of a fuller evaluation. One limitation in our reanalysis (as well as the initial analysis) reflects the underlying assumptions associated with a single summary measure of average formaldehyde exposure based on one to three samples collected during the three week period prior to biological sampling for each study subject. It is unknown whether these exposure measurements reflect long-term exposure levels; however, the expected timeframe for exposures to impact the reported blood parameters may be fairly recent.

Indicators of hematotoxicity

Zhang et al. (2010) reported lower WBC, lymphocyte, granulocyte, platelet, RBC, and monocyte counts in the exposed workers compared to the unexposed workers. If these differences in fact were due to formaldehyde exposure, we would expect to see exposure-dependent differences in these blood parameters across the nearly seven-fold range (0.4–2.7 ppm, excluding the highest value 5.6 ppm) of mean measured individual exposures among exposed workers. Leukemogenic effects, as seen with benzene and alkylating agents, may not correlate closely with exposure, but rather reflect individual genetic predispositions. However, these factors would be expected to be equally distributed between valid comparison, i.e. exposed and unexposed, groups. Although blood parameter values were lower for workers in the formaldehyde exposed group compared with the control workers overall,

differences for granulocyte, platelet, and WBC counts were greater for the workers exposed to formaldehyde concentrations <1.3 ppm than for workers exposed to formaldehyde concentrations ≥1.3 ppm (Figure 2). Given that we would also expect to observe consistent declines in these relationships across levels of exposure intensity among the exposed workers, we performed regression analyses among the exposed workers only (Supplemental Table 2). There was a clear and consistent lack of any association with formaldehyde. That sex and/or smoking were associated with the blood parameters – and in some cases statistically significantly so – suggests that true associations with formaldehyde, if present, would be suggested as well.

Differences in blood parameters are not themselves indicators of leukemia risk. Unusually high or low blood parameter values typically are signs and symptoms of other conditions or diseases. In conjunction with other diagnostic tests, blood count data are used in the clinical evaluation of existing leukemia (American Cancer Society 2016). However, the range of values for exposed and unexposed workers were similar, and no obvious effect of formaldehyde exposure can be seen (Supplemental Table 3). In addition, the mean and maximum values for monocytes (in particular) were higher in unexposed men than exposed men; however, any clinical significance of these results is unlikely and conclusions cannot be drawn from such a small sample size. It would be remarkable if modest differences in these parameters seen in cross-sectional samples of any population were actually predictive of leukemia risk.

Measurements of eosinophils and basophils, components of white blood cells, were not available in the data provided. Although counts of lymphocytes and monocytes were measured, it does not appear that granulocytes were identified and counted by type: eosinophils, basophils, and neutrophils.

Other factors can influence WBC counts, including infections, immune system disorders, and smoking. Smokers consistently have higher WBC counts than non-smokers and the WBC counts increase with smoking level (Sunyer et al. 1996). Smokers may have elevated hemoglobin consequent to an increase in carboxyhemoglobin levels and some increase in neutrophils, but this change in hemoglobin does not explain smokers' increased risk of AML or MDS. Higher WBC counts are also associated with coronary heart disease deaths, independent of the effects of smoking on heart disease (Brown et al. 2001). Dietary factors that influence blood parameters were also unmeasured, for example vitamin B12 or folate deficiency, which are associated with low WBC counts. Future studies should address the limitations of the Zhang et al. (2010) study, including small sample size; poorly controlled comparator populations (since other factors such as workplace stress or differences in genetic predisposing factors could contribute to the subtle differences reported between groups); and temporally-remote toxic endpoints, e.g. a few months to several years for leukemia to develop following exposure to benzene or oncolytic agents.

Aneuploidy

The identification of several serious methodological problems with the original study (Speit et al. 2010; Kuehner et al. 2012; Gentry et al. 2013; Albertini & Kaden 2017) already cast serious doubt on the validity of the findings, specifically with respect to monosomy 7 and trisomy 8, which are genetic anomalies claimed by Zhang et al. (2010) to indicate the biological plausibility that formaldehyde causes leukemia, and presumably AML specifically, as these aneuploidies may not be associated with other myeloid leukemias. As noted above, and as outlined by Zhang et al. (2010), there is considerable uncertainty in drawing conclusions based on analysis of aneuploidies. These are compounded given the methodological issues and resulting loss of study participant data due to failing to meet (or come close to) the counting criteria required by the study protocol. Consistent with methods recently advocated for visualizing data and elucidating bias (Lash et al. 2014) – in this case, over-interpretation bias – we chose to graphically plot the individual aneuploidy results (Figure 3), indicating for which individuals the counting rules were met, by individual formaldehyde exposure estimate. These graphs reinforce the broader conclusion that no confident interpretation of these findings can be made with respect to the possible role, if any, of formaldehyde in causing these aneuploidies. By extension, this illustrates the importance of transparency in study methods, quality control measures, and skepticism in causally interpreting ecological correlations. A full evaluation of the available data provides no basis for concluding that formaldehyde exposure causes leukemia and AML specifically.

The study participants and their data originally used by Zhang et al. (2010) to evaluate correlations between groups of formaldehyde-exposed and unexposed workers and several blood parameters and aneuploidies also have been included in further studies published by Lan et al. (2015) and Bassig et al. (2016). The blood samples used in these evaluations were collected prior to 2009. Lan et al. (2015) expanded the genetic analysis to evaluate frequency of monosomy, trisomy, and tetrasomy, as well as structural changes, for all 24 chromosomes. They also increased the number of subjects for which CFU-GM progenitor cells were cultured from blood samples collected in 2006 and stored for many years (Albertini & Kaden 2017), resulting in a total of 29 formaldehyde-exposed and 24 unexposed workers. The investigators used the same OctoChrome FISH protocol (Zhang et al. 2005) and again reported that at least 150 metaphases per slide were scored for subjects included in this report, as was erroneously stated in their earlier report (Zhang et al. 2010); however, we do not have access to these additional data to verify that the counting rules required by the protocol were followed. Although the analysis by Lan et al. (2015) offered an opportunity to address the critiques of others (Speit et al. 2010; Gentry et al. 2013), the authors offered insufficient details regarding their actual methods to verify any improvements. For example, it is unknown if the results from the 10 exposed workers and 12 controls were re-used or if new cells were cultured. These raise serious doubt regarding the validity of the reported findings (Lan et al. 2015). Nevertheless, the authors interpret their findings as "further evidence that leukemia-related cytogenetic changes can occur in the circulating myeloid progenitor cells of healthy workers exposed to FA, which may be a

potential mechanism underlying FA-induced leukemogenesis" (Lan et al. 2015). However, due to the potentially overlapping and, therefore, non-independent study sample, the results from this study cannot be relied upon to replicate or validate the results from the Zhang et al. (2010) study, and potentially propagate the ecological bias.

Lan et al. (2015) acknowledged two limitations. First, they noted the possibility that chromosomal abnormalities detected in CFU-GM may have arisen during the 14-d cell in vitro culture period, rather than being formed in the bone marrow in vivo and present in the circulating myeloid progenitor cells in the study subject. This criticism has been noted by others (Speit et al. 2010; Gentry et al. 2013; Albertini & Kaden 2017). The authors address this criticism by stating that if the abnormalities arose during the 14 d cell in vitro culture period, then workers exposed to formaldehyde would still exhibit a "greater tendency" to develop abnormalities during cell growth compared with control workers unexposed to formaldehyde, i.e. there is still a significant association with formaldehyde, and such events also "support the leukemogenic potential of FA." However, no analyses to establish a relationship between the reported effects and individual formaldehyde exposure were presented.

Second, Lan et al. (2015) stated that formaldehyde exposure–response analyses were not conducted due to a narrow range of the intensity of formaldehyde. They noted that "further studies of populations exposed to a wider range of FA concentrations are needed to address dose–response in vivo." We note, however, that the range of formaldehyde exposures reported for the workers was relatively large and to relatively high average intensities, to which human populations are rarely exposed today in the US or Europe, even in occupational settings. The US Occupational Safety and Health Association (OSHA) permissible exposure limit (PEL) is 0.75 ppm (8-h TWA) and the American Council of Government and Industrial Hygienists (ACGIH) has adopted a threshold limit value ceiling (TLV-C) limit of 0.3 ppm. In fact, Lan et al. (2015) reported more than a three-fold difference in values between the 90th and 10th percentiles of the 29 exposed workers (2.61 ppm and 0.78 ppm, respectively), while Zhang et al. (2010) reported a similar four-fold difference in values between the 90th and 10th percentiles of 43 exposed workers (2.51 ppm and 0.63 ppm for the 90th and 10th percentiles of exposed workers, respectively). Similar median exposures were reported as well: 1.38 ppm for 29 exposed workers in Lan et al. (2015) and 1.28 ppm for 43 exposed workers in Zhang et al. (2010). Although these ranges may be adequate to evaluate exposure–response associations, the small sample size still may limit the ability to detect any true exposure–response relationships.

Lan et al. (2015) reported confirming the earlier finding of formaldehyde-associated monosomy 7, and also reported an increased frequency of trisomy 8 that was not statistically significant; however, the study population was not independent and the same blood samples were used to culture CFU-GM metaphases. Again, replication, or confirmation would require similar analyses conducted in other individuals or populations exposed to formaldehyde that are not already part of the study.

Conclusions

The IARC has reported that mechanistic data can be pivotal when the human data are not conclusive for carcinogenicity. This certainly remains true, although the epidemiology addressing occupational formaldehyde exposure and acute myeloid leukemia risk has improved since the IARC Working Group review of the evidence for formaldehyde carcinogenicity in 2009 (IARC 2012). Evidence available since the IARC review includes updated studies of the British chemical workers cohort: "Our results provide no support for an increased hazard of myeloid leukemia…" (Coggon et al. 2014) and US garment workers: "We continue to see limited evidence of an association between formaldehyde and leukemia. However, the extended follow-up [of the US garment workers] did not strengthen previously observed associations" (Meyers et al. 2013). From the largest study to date of over 15,000 incident acute myeloid leukemia cases and exposure to occupational exposure to solvents, no association was seen with formaldehyde exposure after adjusting for solvent exposure and ionizing radiation (Talibov et al. 2014). Furthermore, a re-analysis of US industrial workers exposed to formaldehyde (Beane Freeman et al. 2009) concluded, "Findings from this re-analysis do not support the hypothesis that formaldehyde is a cause of AML" (Checkoway et al. 2015). Taken as a whole, the epidemiological evidence from the most recent analyses and follow-up of available cohorts provides little if any evidence of a causal association between formaldehyde exposure and AML.

As the animal toxicological data are negative, a third line of evidence – mechanistic data – remains to be considered. The main cluster of studies published to date that evaluate hypothesized mechanisms are primarily based on the same biological samples analyzed and reported here (Zhang et al. 2010; Hosgood et al. 2013; Lan et al. 2015; Bassig et al. 2016). The additional evaluation of the underlying data including individual average measurements of formaldehyde exposure, however, demonstrates no association between level of formaldehyde exposure among the "exposed" workers and any of the blood parameters. This further challenges the utility of the Zhang et al. (2010) study and its progeny for elucidating potential formaldehyde leukemogenicity. All of the modes or mechanisms of action that have been proposed involve an impact on circulating blood cells, and not on the bone marrow, and how differences observed between groups might lead to AML has not been determined.

A direct genotoxic effect on the bone marrow, resulting in an impact on circulating cells, has been all but disproved (Lu et al. 2011; Moeller et al. 2011; Yu et al. 2015; Lai et al. 2016) based on the inability of exogenous formaldehyde to move beyond the portal of entry. The remaining hypothesized mechanisms of action involve an impact on circulating stem cells at the portal of entry. While Zhang et al. (2010) proposed that formaldehyde exposure leads to aneuploidy, the results from the current analyses indicate that exogenous formaldehyde exposure is not associated with the aneuploidies examined. Therefore, while Zhang et al. (2010) has been cited heavily to support the biological plausibility of formaldehyde as a cause of human leukemia, fuller analysis of the original study data verifies methodological limitations with respect to

monosomy 7 and trisomy 8, while demonstrating no association between individual exposure levels and several blood parameters among those occupationally exposed to formaldehyde. Moreover, a true aneugenic effect would also be seen at high concentrations used in vitro, and independently of the cell line used. Speit et al. (2010) attempted to replicate the in vitro effects reported by Zhang et al. (2010) using a different cell line and reported formaldehyde did not induce aneuploidy, while two positive controls (colcemid and vincristine) did induce aneuploidy. Separately, Kuehner et al. (2012) reported that colony forming ability was not reduced in myeloid progenitor cells in the presence of formaldehyde. Kuehner et al. (2013) reported that the gene expression profile of formaldehyde does not resemble that of known aneugens and more closely resembles that of known clastogens. Therefore, IARC's interpretation of the Zhang et al. (2010) study and the implications on the formaldehyde hazard classification should be reconsidered in light of the fuller evaluation of all of these data, and the updated EPA IRIS report should reflect the limited inferential value of the Zhang et al. (2010) study or any of its progeny (Hosgood et al. 2013; Lan et al. 2015; Bassig et al. 2016) until the scientific validity of each can be demonstrated. In particular, unmeasured factors – including workplace factors – that are distributed differently between the exposed and unexposed workers may explain differences noted in blood parameters and aneuploidies in the original results. IARC (2012) called for the replication of the Zhang et al. (2010) study. We suggest it be replicated using a new study population, actual measured formaldehyde exposures, and valid laboratory tests not subject to methodological problems such as deviation from protocol standards or complicated by questions of the origin (i.e. in vivo versus in vitro) of the effects.

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Declaration of interest

Authors: The design, analyses, presentation of results, interpretations and conclusions reflected in this paper are solely those of the authors. The employment affiliations of each of the authors is as shown on the cover page. We believe that there are no conflicts of interest for any of the authors to disclose related to the work performed in preparing and submitting this manuscript. Most of the authors (Mundt, Gallagher, Dell and Gentry) are full-time employees of Ramboll Environ US Corporation, and conducted work related to this paper as part of their normal employment. Ramboll Environ US Corporation is a consulting firm providing services in environmental and health sciences matters to private firms, trade organizations, and government agencies. Dr. Boffetta (Professor of Medicine, Hematology and Medical Oncology; Professor of Oncological Sciences and Professor of Environmental Medicine & Public Health at the Icahn School of Medicine at Mount Sinai) provided advice on the approach and design of the statistical analysis of the data, the interpretation of the results, and the preparation of the manuscript as an independent consultant to Ramboll Environ with fee for service. Dr. Natelson (Hematologist/Oncologist at Houston Methodist Hospital) provided assistance interpreting the hematological data and technical review on the manuscript and its content as an independent consultant, pro bono.

The authors had sole responsibility for the analyses performed, the interpretations made, conclusions drawn and the writing of the paper, which may not necessarily reflect the views of the sponsor. None of the authors has appeared as expert in any formaldehyde litigation or involved with or appeared in regulatory proceedings related to the contents of this paper. It is anticipated, however, that regulatory authorities will consider the contents of this study in making regulatory decisions relevant to the carcinogenicity of formaldehyde.

Presentation of findings: The analyses presented in this paper were based on data provided by the National Cancer Institute, as acknowledged above, and results have not been previously published. However, some of the findings reported here were presented to EPA IRIS staff on November 7, 2016 and the slides related to this publication (specifically slides 14–16) are posted on the EPA website, IRIS Calendar, Meetings Requested by Specific Members of the Public (https://cfpub.epa.gov/ncea/iris2/events.cfm).

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Supplemental material

Supplemental data for this article can be accessed here.

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"Formaldehyde, Hematotoxicity, and Chromosomal Changes" - Letter

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To the Editor:

Zhang et al. (1) compared hematological parameters and prevalence of aneuploidy (monosomy 7 and trisomy 8) between groups of workers exposed to "relatively high levels" of formaldehyde and those occupationally unexposed. The International Agency for Research on Cancer (IARC) identified this study as "particularly relevant to the discussions regarding sufficient evidence" in classifying formaldehyde as leukemogenic (2). Similarly, the EPA IRIS Draft Toxicological Review of Formaldehyde noted that this study's findings "support the biological plausibility of formaldehyde effects on the hematopoietic system" and provide "the best evidence for bone marrow toxicity, where they report not only a reduction in white blood cell counts, but reductions in cell counts of all the blood cells, as well as increased mean cell volume" (3). However, important methodological limitations have been reported, including the lack of evidence that group differences in aneuploidy are significant to leukemogenesis, that personal monitoring data were collected but not analysed and presented, and failure to adhere to the study protocol. Additionally, the cross-sectional study design precludes identification of "changes" or "reductions" as claimed, as individual outcome parameters were measured only once (4).

NCI recently provided us the mean (but not raw) formaldehyde measurements described by Zhang et al. (1). Exposure-response analyses adjusting for sex and smoking found no relationship between formaldehyde exposure level and any of the hematological parameters. Statistical analysis of monosomy 7 and trisomy 8 prevalence by formaldehyde exposure was impossible after applying standard 2% background rates and strictly adhering to the study protocol criterion of counting >150 cells (clinical evaluations commonly require 200-400). Even disregarding minimum counting criteria, no association between concentration and aneuploidy was seen among formaldehyde exposed workers (4).

Findings based on these new analyses of original study data contradict the conclusions and interpretation of Zhang et al. (1) and progeny publications using the same data. In one of these, Lan et al. (5) claimed it was "a relatively narrow range of [formaldehyde] exposure that precluded their assessment of exposure-response relationships". However, the 10th-90th percentile formaldehyde exposure concentration range reported by Zhang et al was 0.78-2.51 ppm (1), extending more than threefold above the US OSHA PEL of 0.75 ppm. Reliance on Zhang et al. (1) to support biological plausibility of an association between formaldehyde exposure and leukemia should be tempered until its scientific validity can be verified and its findings properly replicated, the need for which was acknowledged in the Draft Formaldehyde Assessment (3).

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Formaldehyde Exposure and Mortality Risks From Acute Myeloid Leukemia and Other Lymphohematopoietic Malignancies in the US National Cancer Institute Cohort Study of Workers in Formaldehyde Industries

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Objectives: To evaluate associations between cumulative and peak formaldehyde exposure and mortality from acute myeloid leukemia (AML) and other lymphohematopoietic malignancies. **Methods:** Cox proportional hazards analyses. **Results:** Acute myeloid leukemia was unrelated to cumulative exposure. Hodgkin lymphoma relative risk estimates in the highest exposure categories of cumulative and peak exposures were, respectively, 3.76 (P_{trend} = 0.05) and 5.13 (P_{trend} = 0.003). There were suggestive associations with peak exposure observed for chronic myeloid leukemia, albeit based on very small numbers. No other lymphohematopoietic malignancy was associated with either chronic or peak exposure. **Conclusions:** Insofar as there is no prior epidemiologic evidence supporting associations between formaldehyde and either Hodgkin leukemia or chronic myeloid leukemia, any causal interpretations of the observed risk patterns are at most tentative. Findings from this re-analysis do not support the hypothesis that formaldehyde is a cause of AML.

Formaldehyde is environmentally and biologically ubiquitous. Major occupational exposure sources include manufacturing of construction materials, plastics, and garments. Cigarette smoking, consumer products including personal care products and some medications, and ambient air pollution are common nonoccupational sources.[1,2] Formaldehyde is also produced endogenously and is an essential intermediate in the biosynthesis of purines, thymidine, and various amino acids.[3] Thus, formaldehyde is present in small quantities in all body tissues. Exogenous formaldehyde is rapidly metabolized at the site of entry (typically the upper respiratory tract). There is consistent evidence that exogenous formaldehyde cannot reach distant organs including the bone marrow.[4–7]

Cancer risks associated with formaldehyde exposure have been investigated in occupational cohort and community-based case–control studies. The occupational cohort studies generally provide higher-quality evidence than population-based case–control studies—primarily due to better exposure data and a greater potential for higher and more sustained levels of formaldehyde exposure.[8] In 2009, the International Agency for Research on Cancer Working Group concluded that "There is sufficient evidence in humans for the carcinogenicity of formaldehyde. Formaldehyde causes cancer of the nasopharynx and leukaemia."[9(p430)] Baan et al summarized the findings of the Working Group meeting and reported that "The Working Group concluded that, overall, there is sufficient evidence for leukaemia, particularly myeloid leukaemia."[10(p1144)] Despite the clear language regarding causation, the Volume 100F monograph reported that the consensus was based on the small majority of the working group who held the view that the evidence for leukemia was sufficient while a minority of the working group found the evidence for leukemia to be limited. Subsequently, the National Institute of Environmental Health Sciences National Toxicology Program changed the classification of formaldehyde from "anticipated to be carcinogenic in humans" as listed in the Second Report on Carcinogens (RoC) to "known to be a human carcinogen" in the 12th RoC.[11] (Each revision of the RoC is cumulative and includes previous substances as well as newly reviewed substances. The 13th RoC, released in October 2014, contains 243 substance profiles.) The change in classification from anticipated carcinogen to known carcinogen was based on "consistent findings of increased risks of nasopharyngeal cancer, sinonasal cancer, and lymphohematopoietic cancer, specifically myeloid leukemia among individuals with higher measures of exposure to formaldehyde (exposure level or duration), which cannot be explained by chance, bias, or confounding. The evidence for nasopharyngeal cancer is somewhat stronger than that for myeloid leukemia."[11] Findings from one large cohort mortality study of workers from 10 US plants producing or using formaldehyde [12] have been especially influential in the designation by the International Agency for Research on Cancer[9] and the National Institute of Environmental Health Sciences National Toxicology Program[13] of formaldehyde as leukemogenic. This study was begun by the US National Cancer Institute (NCI) in the 1980s in collaboration with the Formaldehyde Institute, and the first results were published in 1986.[14]

Sequential analyses of updated mortality for the NCI cohort[12,15] reported associations of "peak" exposures with myeloid leukemia (ML) and Hodgkin lymphoma (HL), but not with cumulative, average, or frequency of "peak" exposures. Null or very weak associations were observed with cumulative or "peak" exposures and the other specific lymphohematopoietic malignancies (LHMs) including lymphatic leukemia (LL), non-Hodgkin lymphoma (NHL), and multiple myeloma. Acute myeloid leukemia (AML) and chronic myeloid leukemia (CML) were not reported separately in the NCI analyses but were combined as ML.

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Although both AML and CML arise in myeloid stem cells, the risk factors associated with AML and CML differ. Most individuals diagnosed with CML have a gene mutation in the leukemia cells called the Philadelphia chromosome, describing the translocation between chromosomes 22 and 9. The translocation leads to the development of the Bcr-Abl oncogene, and this gene instructs the bone marrow to produce Bcr-Abl tyrosine kinase, leading to the development of CML.[16,17] In addition, the known risk factors for AML—tobacco smoking, exposure to benzene, chemotherapy, or radiation treatment—are not recognized risk factors for CML.[17] High-dose radiation, such as that experienced by survivors of atomic bombs or nuclear reactor accidents, is the only recognized environmental risk factor for CML.[17] These recognized differences in histopathology and in the risk factors for AML and CML raise the question of whether the reported association between formaldehyde exposure and combined MLs reflects an underlying association between formaldehyde exposure and the more plausible specific type of leukemia, AML.

We obtained the data included in the most recent update of the NCI cohort[12] via a Technology Transfer Agreement. Our objectives were to replicate the updated findings reported by Beane Freeman et al[12] and to conduct additional analysis of associations of specific LHM, and especially AML, with peak exposure, using an alternative, more standard definition of peak.

METHODS

We performed analyses to replicate findings reported in the most recent follow-up of the cohort,[12] including the descriptive characteristics of the cohort—the number of workers, person-time, median length of follow-up, race, sex, pay category, the number of deaths, and median age at entry and exit from the study. We also replicated the reported number of workers never exposed to formaldehyde, median and range for estimated formaldehyde 8-hour time-weighted average (TWA8) exposures, cumulative exposure, the number of workers with average intensity levels 1.0 ppm or more, and the number of workers who experienced peak formaldehyde exposures 4.0 ppm or more. Cause-specific mortality among the cohort was compared with race- and sex-specific national mortality rates by age and calendar interval for cause-specific categories of death from the National Institute for Occupational Safety and Health (NIOSH, Atlanta, GA) by computing standardized mortality ratios (SMRs).[18,19] Rates for lymphatic leukemia, ML, AML, and CML were not available through NIOSH and were instead obtained from the Surveillance Epidemiology and End Results Cancer Query Systems (CanQues).[20] The final replication included exposure–response analyses for cumulative, average, and peak exposures with mortality for LHM, using the same exposure metrics defined by Stewart et al.[21] and mortality outcome categories as reported in Beane Freeman et al.[12] Only trivial differences were found.

In the original analysis, peak exposures were defined as estimates of "short-term exposures (generally less than 15 minutes) that exceeded the TWA8 category".[12,21] Workers in jobs not identified as having peak exposure levels that exceeded the TWA8 category were assigned the TWA8 intensity category as their peak exposure. Thus, peaks were defined on a worker-specific relative basis. Moreover, neither frequency nor duration of peaks had been included in the definition of the peak exposure metric previously (eg, at least 1 year of employment in jobs likely experiencing more than 4 ppm exposure for 15 to 60 minutes at least weekly). For our reanalyses, we redefined peak exposures on an absolute scale, that is, at least 1 continuous month of employment in jobs identified in the original exposure characterization as likely having short-term exposure excursions of 2 ppm or more to less than 4 ppm or 4 ppm or more on a weekly or daily basis.[21] Our definition of peak exposure did not include employment in jobs likely experiencing (1) short-term excursions more than 0 ppm and less than 2 ppm; (2) short-term excursions identified as occurring as frequently as hourly; and (3) short-term excursions identified as occurring as infrequently as monthly.

We applied Cox proportional hazards models to estimate exposure–response relations for both cumulative and the newly derived absolute peak exposures (Stata Statistical Software, College Station, TX). These methods produce statistically similar results to Poisson regression,[22] and both methods can accommodate time-dependent treatment of exposure variables. Replication of previous results allowed us to extend our analysis to examine the robustness of previously observed associations between peak exposure (as originally defined) and mortality from specific LHMs, including subtypes of leukemia as well as original analyses of AML and CML risk.

Peak exposure was treated in a time-dependent manner such that subjects accrued person-time in the non–peak exposure category until the start of their first peak exposure job, after which they accrued person-time in that specific peak exposure category. Because of the time-varying nature of peak exposure, in which study subjects may also transfer from job assignments with peak exposures to subsequent job assignments without peak exposures, we also conducted a sensitivity analysis to evaluate duration of time in jobs with short-term excursions 2 ppm or more.

Cumulative formaldehyde exposure was modeled categorically, with cut points based on approximate quartiles of exposure (rounded to the nearest half fraction) for the full cohort. Because of the small number of HL (n = 5) and subtypes of ML deaths (n = 4 AML and n = 2 CML) in the lowest exposure quartile (ie, less than 0.05 ppm-years), the first two quartiles of exposure were combined to form a new referent category (less than 0.5 ppm-years). Cumulative exposure was also treated in a time-dependent manner, with exposure accruing on a yearly basis.

We did not conduct analyses according to average exposure intensity because the previous findings for ML with respect to average intensity were unremarkable (as were the findings for cumulative exposure). Moreover, cumulative exposure is the conventional exposure metric used for risk assessment of chronic diseases, such as cancer, and the default policy for regulatory quantitative risk assessment assumes proportionality of cancer risk with cumulative exposure. Average exposure intensity is also correlated with cumulative exposure, which is the sum of average intensity in job times duration in job over all jobs in an employee's work history.

All Cox models of peak and cumulative exposures used attained age as the time scale and controlled for sex, race (white or other), and pay category (salary, ever wage, or unknown).

Analyses were conducted for NHL, chronic lymphocytic leukemia (CLL), HL, multiple myeloma, ML, AML, and CML, and combining all leukemias. We included CLL in the NHL grouping because CLL has been classified as NHL since 2001.[23,24]

On the basis of observations of workers exposed to high concentrations of benzene, AMLs are expected to occur within 10 or at most 15 years since first exposure.[25–27] Therefore, peak exposures occurring up to 10 years preceding death would be particularly relevant for AML etiology. We also performed separate Cox models to lag exposure by 1, 2, or 5 years to allow for disease latency intervals. These analyses were conducted for the entire cohort and separately for the subset of 16,306 employed for 1 year or more to eliminate possible confounding by unmeasured risk factors or underlying health and risk differences associated with short-term employment. A disproportionate number of HL and AML deaths in the reference group was lost when the analyses were restricted to cohort members employed at least 1 year—five of nine HLs (56%) and 11 of 17 AMLs (65%) were lost in the cumulative exposure analysis; and 7 of 15 HLs (47%) and 9 of 21 AMLs (43%) were lost in the peak exposure analysis. To stabilize the referent group, we combined the first two quartiles of exposure into a new referent category.

Because job histories were available only through 1980, exposure histories were incomplete for the 3434 persons (13.4%) known

to have worked after that date. In addition, no information was available on formaldehyde exposure for any work history before entry into the cohort or subsequent to leaving the industry. We performed sensitivity analyses by separately analyzing survival for the study subjects with complete work history and ending follow-up in 1985 and by assigning exposure of the most recent job until the age of 65 years or the end of follow-up for those with truncated work history.

We also performed separate sensitivity analyses in which we assumed that all 21 deaths coded as "acute leukemia, NOS" (ICD-8 207.0) were either AML deaths or ALL deaths, as well as analyses that evaluated time since first exposure to formaldehyde and time since first exposure to peak 4 ppm or more, consistent with results reported in the online supplementary tables by Beane Freeman et al.[12] Only four deaths were reported as "chronic leukemia, unspecified" on death certificates (compared with 13 CMLs and 32 CLLs), and therefore we did not conduct additional analyses reclassifying these into assumed specific categories.

RESULTS

Descriptive features of the full cohort, the subset employed 1 year or more, and the subset employed less than 1 year are summarized in Table 1.

A total of 25,619 formaldehyde workers were followed from year of first employment at the facility (1930 to 1966) or year of cohort identification (1934 to 1958), whichever was later, through death, loss-to-follow-up, or December 31, 2004, whichever was earliest. We calculated 997,514 person-years compared with 998,106 as reported by Beane Freeman et al.[12] Of the total 25,619 workers, 3478 (13.6%) worked in jobs with peaks 2 ppm or more to less than 4 ppm, and 2907 (11.3%) had jobs with peaks 4 ppm or more.

TABLE 1. Descriptive Statistics Comparing the Full Cohort (n = 25,619) and Workers Employed for 1 Year or Longer (n = 16,306)

Variable	Full Cohort (n = 25,619)	Workers Employed 1 Yr or More (n = 16,306)	Workers Employed Less Than 1 Yr (n = 9,313)
Race (%)			
White	23,758 (92.7)	15,148 (92.9)	8,610 (92.5)
Nonwhite	1,861 (7.3)	1,158 (7.1)	703 (7.6)
Sex (%)			
Male	22,493 (87.8)	14,310 (87.8)	8,183 (87.9)
Female	3,126 (12.2)	1,996 (12.2)	1,130 (12.1)
Pay status (%)			
Hourly	20,116 (78.5)	11,970 (73.4)	8,146 (87.5)
Salaried	4,600 (18.0)	3,948 (24.2)	652 (7.0)
Unknown	903 (3.5)	388 (2.4)	515 (5.5)
Duration of follow-up, yrs			
Mean (standard deviation)	38.9 (13.9)	39.0 (13.2)	38.8 (15.0)
Median (range)	41.8 (0.1–66.9)	41.8 (0.1–66.9)	41.8 (0.1–65.2)
25th percentile	31.8	31.9	31.6
75th percentile	48.5	47.9	49.4
Duration of employment, yrs			
Mean (standard deviation)	9.0 (11.3)	13.9 (11.5)	0.4 (0.3)
Median (range)	2.6 (>0.0–47.6)	11.1 (1.0–47.6)	0.3 (>0.0–<1.0)
25th percentile	0.5	3.1	0.2
75th percentile	16.5	23.5	0.6
Age at start of follow-up, yrs			
Mean (standard deviation)	29.1 (10.2)	30.4 (10.5)	26.8 (9.1)
Median (range)	26.0 (8.1–82.7)	27.7 (8.1–82.7)	23.7 (15.2–82.6)
25th percentile	21.1	22.1	20.0
75th percentile	34.9	37.0	30.9
Age at end of follow-up, yrs			
Mean (standard deviation)	68.0 (13.3)	69.5 (12.4)	65.6 (14.4)
Median (range)	69.3 (15.3–102.0)	70.5 (17.4–102.0)	67.2 (15.3–102.0)
25th percentile	61.6	62.7	59.8
75th percentile	76.9	77.9	75.2
Cumulative exposure, ppm-yr			
Mean (standard deviation)	3.2 (8.4)	4.9 (10.1)	0.2 (0.3)
Median (range)	0.4 (0.0–107.5)	1.4 (0.0–107.5)	0.1 (0.0–3.4)
25th percentile	0.04	0.3	0.01
75th percentile	2.4	5.0	0.2
Peaks (%)			
≥2–<4 ppm peak	3,478 (13.6)	2,712 (16.6)	766 (8.2)
≥4 ppm peak	2,907 (11.3)	2,631 (16.1)	276 (3.0)
Study subjects with complete work history (%)	22,185 (86.6)	12,872 (78.9)	9,313 (100.0)

We replicated closely the SMR findings reported in the original analysis and added analyses for AML and CML separately (Table 2). When all deaths from "Acute Leukemia, NOS" were assumed to be AML, the AML SMRs increased from 0.80 (95% confidence interval [CI], 0.56 to 1.14, based on 30 deaths) to 0.94 (95% CI, 0.71 to 1.25 based on 49 deaths) for the formaldehyde-exposed group and from 0.93 (95% CI, 0.25 to 2.37, based on four deaths) to 1.00 (95% CI, 0.45 to 2.23, based on six deaths) for the group not exposed to formaldehyde (results not shown). Thus, the deficit of AMLs is unlikely due to ambiguous coding of acute leukemia deaths.

All Leukemias

No association between cumulative formaldehyde exposure and mortality from all leukemias combined was observed for the entire cohort (Table 3).

Nevertheless, risks were elevated among those employed for 1 year or more, regardless of cumulative exposure category, due to the large loss of leukemia cases in the referent group (27 cases worked less than 1 year)—hazard ratio (HR) = 2.44; 95% CI, 1.08 to 5.51 for those with cumulative exposures of 0.5 to less than 2.5 ppm-years and HR = 2.49; 95% CI, 1.13 to 5.49 for those with 2.5 ppm-years or more (P_{trend} = 0.04; Table 3).Peak exposures 2.0 ppm or more to less than 4 ppm (HR = 2.23; 95% CI, 1.34 to 3.72) and 4.0 ppm or more (HR = 2.07; 95% CI, 1.22 to 3.49) were associated with all leukemias, and similar associations were seen among those employed for 1 year or more (HR = 2.46; 95% CI, 1.29 to 4.67 and HR = 2.45; 95% CI, 1.32 to 4.52, respectively) (Table 4).

Myeloid Leukemias

Myeloid leukemia (all types combined) was not associated with cumulative formaldehyde exposure in the entire cohort. There was, however, a modest, but not statistically significant, association of cumulative exposure and ML among workers employed 1 year or more (Table 3). Peak exposure of 2.0 ppm or more to less than 4 ppm was associated with ML in the full cohort (HR = 2.09; 95% CI, 1.03 to 4.26) and similarly among those employed 1 year or more (HR = 2.49; 95% CI, 1.01 to 6.15) (Table 4). HRs for peaks of 4.0 ppm or more were weaker, but still elevated, and trends were not statistically significant (ie, P_{trend} = 0.06 and 0.08, respectively).

CML and AML

The association seen with peak exposure and ML was examined by specific subtype of ML, that is, AML and CML; however, numbers were small, and therefore HR estimates were imprecise. HR estimates for CML among the full cohort were elevated for peak exposure 2.0 ppm or more to less than 4.0 ppm (HR = 2.62; 95% CI, 0.64 to 10.66) and 4.0 ppm or more (HR = 3.07; 95% CI, 0.83 to 11.40). For AML, risk estimates were considerably lower and did not increase at the highest peak formaldehyde levels. The AML findings were only minimally changed when 21 deaths from "acute leukemia, NOS" all were assumed to be AML.

Analysis of time since first and time since last peak exposure revealed that, among the 13 of 34 AML deaths in the full cohort with peak exposures more than 2.0 ppm, only four worked in jobs with peaks within the 20 years preceding death, and only one occurred (similar to expected) within the typical AML latency window of 2 to 15 years.

Hodgkin Lymphoma

Of the LHMs, HL was most strongly and consistently associated with both cumulative (Table3) and peak (Table4) formaldehyde exposures. For the full cohort, the HRs for HL were 2.52 (95% CI, 0.93 to 6.83) and 3.11 (95% CI, 1.16 to 8.34) for cumulative exposure 0.5 to less than 2.5 ppm-years and 2.5 ppm-years or more, respectively; HR estimates (95% CI) for peak exposure categories were 2.18 (0.77 to 6.19) and 3.38 (1.30 to 8.81), respectively,for peak categories 2 ppm or more to less than 4 ppm and 4 ppm or more, respectively. Similar results were observed for workers employed for 1 year or more.

Other LHMs

None of the other LHMs was associated with either cumulative or peak exposure (Tables 3 and 4).

The results presented in Tables 3 and 4 were not materially different when we applied exposure lags (1, 2, or 5 years), adjusted for total employment duration, adjusted for exposure confidence score,[21] or when follow-up was truncated as of 1985 (ie, limiting the exposure to the years for which work history/exposure data were available) (results not presented but available on request). Results were only minimally changed when we restricted analyses to cohort members with complete work histories and ended follow-up in 1985, or when we assigned people with incomplete work/exposure history to the exposure of their final job until the age of 65 years or end of follow-up (results not presented but available on request).

DISCUSSION

The NCI study of occupational formaldehyde exposure has been influential in the recent classification of formaldehyde as a human leukemogen. The primary objectives of our reanalyses of these data were to determine the robustness of the findings to alternative exposure classification schemes, especially for peak exposures, and to evaluate whether formaldehyde exposure metrics were associated specifically with AML mortality. In the original analysis conducted by the NCI investigators, peak was defined on a relative basis, with respect to individual workers' exposure histories. This approach to defining peaks complicates data interpretation and risk assessments that are ultimately applied to set occupational and environmental exposure standards. The alternative approach that we applied defined peaks in terms of absolute exposure intensity and duration and also treated peaks as a time-varying exposure. This approach is a decided strength of the re-analysis because it permits direct comparisons among similar studies and is applicable to risk assessment. As for formaldehyde exposure and AML mortality, no results specific to AML—the type of leukemia most plausibly related to chemical exposures—had been presented in any of the previous publications on this cohort.

One general limitation of the data from this cohort is that job assignments were not documented beyond the initial study end date; thus, exposures could not be estimated for years worked after 1980. To overcome this limitation, Beane Freeman et al[12] performed sensitivity analyses to evaluate the effect of unknown exposures after 1980. We also evaluated the effect of unknown exposure by assuming that exposure continued in the last assigned job held until the age of 65 years, death, or end of follow-up. We also analyzed mortality for the cohort members with complete work history records and ending follow-up as of 1985. None of these approaches generated different results, suggesting that exposures in later years, which would be expected to be low relative to earlier years, were not determinants of mortality risks.

Another inherent limitation of this study is that despite its large overall size and nearly 1 million person-years of follow-up, there is a relatively small number of AML deaths observed among individuals employed for more than 1 year and most highly exposed to formaldehyde. Acute myeloid leukemia is the specific ML plausibly associated with chemical risk factors, such as benzene[9] and antineoplastic agents.[28] Furthermore, few of the employees who died of AML had any peak exposures (as originally defined or as we redefined it here), and nearly none had peak exposures within a reasonable time window of latency. For this reason, extending follow-up of mortality will not be helpful for shedding light on AML associations with peak exposure because the cohort is now 35 years since

TABLE 2. Replication of Mortality From Lymphohematopoietic Malignancies Among a Cohort of US Workers Nonexposed and Exposed to Formaldehyde, Follow-Up Through 2004

	Beane Freeman et al (2009)[12]*				Current Analysis 2014†			
	Nonexposed (n = 3,108)		Exposed (n = 22,511)		Nonexposed (n = 3,136)		Exposed (n = 22,483)	
Cause of Death (ICD-8 Codes)	Observation	SMR (95% CI)	Observation	SMR (95% CI)	Observation	SMR (95% CI)	Observation	SMR (95% CI)
Lymphohematopoietic malignancies (200–209)†	33	0.86 (0.61–1.21)	286	0.94 (0.84–1.06)	NC†	—	NC†	—
NHL (200, 202)‡	12	0.86 (0.49–1.52)	94	0.85 (0.70–1.05)	12	0.90 (0.51–1.59)	94	0.83 (0.68–1.01)
Hodgkin disease (201)	2	0.70 (0.17–2.80)	25	1.42 (0.96–2.10)	2	1.04 (0.13–3.74)	25	1.34 (0.91–1.99)
Multiple myeloma (203)	11	1.78 (0.99–3.22)	48	0.94 (0.71–1.25)	11	1.82 (1.01–3.29)	48	0.93 (0.70–1.24)
Leukemia (204–207)	7	0.48 (0.23–1.01)	116	1.02 (0.85–1.22)	7	0.53 (0.25–1.12)	116	1.01 (0.84–1.21)
Lymphatic leukemia (204)§	1	0.26 (0.04–1.82)	36	1.15 (0.83–1.59)	1	0.28 (0.01–1.57)	36	1.14 (0.82–1.57)
Myeloid leukemia (205)§	4	0.65 (0.25–1.74)	44	0.90 (0.67–1.21)	4	0.69 (0.19–1.76)	44	0.86 (0.64–1.16)
AML (205.0)§	NR	—	NR	—	4	0.93 (0.25–2.37)	30	0.80 (0.56–1.14)
CML (205.1)§	NR	—	NR	—	0	—	13	0.97 (0.56–1.67)

*US rates obtained from the National Cancer Institute Surveillance Epidemiology and End Results (SEER) (personal correspondence, Dr Beane Freeman, October 22, 2013).

†US age-, sex-, race-, and calendar-specific mortality rates, 1960 to 2007 obtained from NIOSH. 1960 rates were applied to earlier years. NIOSH rates for ICD-8 204, 205, 208, and 209 were not provided separately. ICD-8 208 is included with other benign and unspecified nature neoplasms. ICD-8 209 is included with all other disease of blood forming organs.

‡The NIOSH rate for NHL also includes ICD, 8th revision, code 275.5.

§SEER CanQues US mortality rates for 1970 to 2009 were used in the Current Analysis (2014) for LL, ML, AML, and CML. 1970 rates were applied to earlier years. Nonwhite workers in the data set were compared with rates for blacks from SEER US mortality rates. The myeloid leukemia rate is the sum of the AML and CML rates. One death was coded to ICD-8 205.9, unspecified myeloid leukemia. Other and unspecified myeloid leukemias are not included in the rate because SEER only provides a combined "other myeloid/monocytic leukemia" category.

AML, acute myeloid leukemia; CI, confidence interval; CML, chronic myeloid leukemia; ICD-8, International Classification of Diseases, 8th Revision; NC, not calculated; NHL, non-Hodgkin lymphoma; SMR, standardized mortality ratio.

TABLE 3. Association Between Cumulative Exposure to Formaldehyde and Death From Lymphohematopoietic Malignancies, Mortality Follow-Up Through 2004

Category of Death (ICD-8 Codes)		Full Cohort (n = 25,619)	Worked ≥1 Y r (n = 16,306)	
Cumulative Exposure (ppm-yr)	No. of Deaths	HR† (95% CI)	No. of Deaths	HR† (95% CI)
NHL (200, 202, 204.1)				
0–<0.5	68	1.0 (referent)	33	1.0 (referent)
0.5–<2.5	33	0.96 (0.63–1.46)	27	0.79 (0.47–1.32)
≥2.5	37	0.77 (0.51–1.16)	37	0.65 (0.40–1.07)
P trend		0.22		0.09
CLL (204.1)				
0–<0. 5	14	1.0 (referent)	6	1.0 (referent)
0.5–<2.5	9	1.21 (0.52–2.81)	6	0.93 (0.29–2.96)
≥2.5	9	0.82 (0.35–1.93)	9	0.81 (0.28–2.37)
P trend		0.69		0.69
Hodgkin lymphoma (201)				
0–<0.5	9	1.0 (referent)	4	1.0 (referent)
0.5–<2.5	8	2.52 (0.93–6.83)	6	2.46 (0.63–9.55)
≥2.5	10	3.11 (1.16–8.34)	10	3.76 (0.99–14.26)
P trend		0.02		0.05
Multiple myeloma (203)				
0–<0.5	34	1.0 (referent)	19	1.0 (referent)
0.5–<2.5	6	0.37 (0.16–0.90)	5	0.27 (0.10–0.74)
≥2.5	19	0.88 (0.49–1.58)	19	0.65 (0.33–1.28)
P trend		0.51		0.29
All leukemia (204–207, excluding 204.1)				
0–<0.5	36	1.0 (referent)	9	1.0 (referent)
0.5–<2.5	23	1.27 (0.75–2.15)	20	2.44 (1.08–5.51)
≥2.5	32	1.29 (0.79–2.10)	32	2.49 (1.13–5.49)
P trend		0.30		0.04
Myeloid leukemia (205)				
0–<0.5	23	1.0 (referent)	7	1.0 (referent)
0.5–<2.5	11	0.98 (0.47–2.03)	9*	1.53 (0.54–4.27)
≥2.5	14	0.94 (0.47–1.86)	14	1.58 (0.59–4.23)
P trend		0.85		0.39
AML (205.0)				
0-<0.5	17	1.0 (referent)	6	1.0 (referent)
0.5-<2.5	7	0.87 (0.36–2.12)	6	1.16 (0.36–3.76)
≥2.5	10	0.96 (0.43–2.16)	10	1.31 (0.44–3.95)
P trend		0.90		0.63
CML (205.1)				
0–<0.5	6	1.0 (referent)	1	1.0 (referent)
0.5–<2.5	3	0.97 (0.24–3.93)	2	2.91 (0.24–35.64)
≥2.5	4	0.92 (0.25–3.36)	4	3.81 (0.36–40.44)
P trend		0.90		0.27

*Includes one death from myeloid leukemia, not specified as acute or chronic.

†Cox proportional hazards model using attained age as the time scale variable, adjusted for sex, race (white or other), and pay category (salary, ever wage, or unknown). Results were comparable to the original results based on the Poisson regression models for specific LHMs, and we additionally conducted specific analyses for AML and CML. Minor differences remaining between results can be attributed to some methodological refinements as well as rounding error (eg, we were only provided data on month rather than exact dates of employment changes).

AML, acute myeloid leukemia; CI, confidence interval; CLL, chronic lymphocytic leukemia; CML, chronic myeloid leukemia; HR, hazard ratio; ICD-8, International Classification of Diseases, 8th Revision; NHL, non-Hodgkin lymphoma.

last known peak exposure, and AMLs increase sharply with older age, independent of exposure. We also explored the 21 deaths identified as "acute leukemia, unspecified" on death certificates; these likely represent some unknown combination of AML and ALL diagnoses (only three ALLs were reported on death certificates, with 5.8 expected, suggesting that ALLs were underreported).

Our HL results, similar to previous reports on this cohort, identified a slight overall excess of HL deaths among exposed workers (Table 2). Five of nine HL deaths in the referent group had worked less than 1 year (Table 3). Furthermore, our reanalyses confirmed associations between different exposure metrics (cumulative and peak) to formaldehyde and HL. Interpretation of the HL

TABLE 4. Association Between Peak (ie, Short-Term Excursions 2 ppm or More to Less Than 4 ppm and 4 ppm or More)* Not Lagged And Death From Lymphohematopoietic Malignancies, Mortality Follow-Up Through 2004

		Full Cohort (n = 25,619)	Worked ≥1 ye a r(= 16,306)	
Category of Death (ICD-8 Codes) Peak Exposure	No. of Deaths	HR† (95% CI)	No. of Deaths	HR† (95% CI)
NHL (200, 202, 204.1)				
No peak‡	98	1.0 (referent)	63	1.0 (referent)
≥ 2.0–<4 ppm	19	0.94 (0.58–1.55)	16	0.93 (0.53–1.61)
≥4 ppm	21	0.98 (0.60–1.58)	18	0.89 (0.52–1.52)
P trend		0.88		0.65
CLL (204.1)				
No peak‡	23	1.0 (referent)	13	1.0 (referent)
≥2.0–<4 ppm	4	0.79 (0.27–2.30)	4	1.07 (0.35–3.32)
≥4 ppm	5	0.95 (0.36–2.52)	4	0.91 (0.29–2.83)
P trend		0.82		0.90
Hodgkin lymphoma (201)				
No peak‡	15	1.0 (referent)	8	1.0 (referent)
≥2.0–<4 ppm	5	2.18 (0.77–6.19)	5	3.50 (1.06–11.56)
≥4 ppm	7	3.38 (1.30–8.81)	7	5.13 (1.67–15.77)
P trend		0.01		0.003
Multiple myeloma (203)				
No peak‡	43	1.0 (referent)	28	1.0 (referent)
≥2.0–<4 ppm	8	0.99 (0.46–2.13)	7	0.98 (0.43–2.28)
≥4 ppm	8	0.95 (0.44–2.06)	8	0.97 (0.43–2.16)
P trend		0.90		0.94
All leukemia (204–207, excluding 204.1)				
No peak‡	48	1.0 (referent)	26	1.0 (referent)
≥2.0–<4 ppm	22	2.23 (1.34–3.72)	16	2.46 (1.29–4.67)
≥4 ppm	21	2.07 (1.22–3.49)	19	2.45 (1.32–4.52)
P trend		0.001		0.002
Myeloid leukemia (205)				
No peak‡	27	1.0 (referent)	14	1.0 (referent)
≥2.0–<4 ppm	11	2.09 (1.03–4.26)	8	2.49 (1.01–6.15)
≥4 ppm	10	1.80 (0.85–3.79)	8	2.03 (0.82–5.03)
P trend		0.06		0.08
AML (205.0)				
No peak‡	21	1.0 (referent)	12	1.0 (referent)
≥2.0–<4 ppm	7	1.71 (0.72–4.07)	5	1.78 (0.61–5.25)
≥4 ppm	6	1.43 (0.56–3.63)	5	1.51 (0.51–4.44)
P trend		0.31		0.37
CML (205.1)				
No peak‡	6	1.0 (referent)	2	1.0 (referent)
≥2.0–<4 ppm	3	2.62 (0.64–10.66)	2	4.83 (0.64–36.42)
≥4 ppm	4	3.07 (0.83–11.40)	3	5.32 (0.81–34.90)
P trend		0.07		0.07

*1 month or more continuous exposure.

†Attained age as the time scale variable, adjusted for sex, race (white or other), and pay category (salary, ever wage, or unknown).

‡Referent group includes study subjects with peaks less than 2 ppm of hourly, daily, weekly, and monthly frequency as well as peaks 2 ppm or more if hourly or monthly frequency.

AML, acute myeloid leukemia; CI, confidence interval; CLL, chronic lymphocytic leukemia; CML, chronic myeloid leukemia; HR, hazard ratio; ICD-8, International Classification of Diseases, 8th Revision; NHL, non-Hodgkin lymphoma.

findings is complicated because there is little epidemiologic support for chemical exposures in the etiology of HL. In particular, increased risk of HL has not been observed in other occupational studies of formaldehyde-exposed cohorts. Coggon et al[29] reported an SMR of 0.70 (95% CI, 0.26 to 1.53) based on six deaths during 1940 to 2000 among more than 14,000 men employed after 1937 in the UK formaldehyde industry. Although follow-up was extended through 2012 in the UK cohort, results were not presented for HL.[30] No increased risk of HL was observed in a recent update of more than 11,000 garment workers followed for mortality from the mid-1950s to 2008 based on four deaths (SMR = 0.95; 95% CI, 0.26 to 2.44).[31]

Hodgkin leukemia is heterogeneous with respect to age at diagnosis and histology. The incidence of HL is described by a bivariate distribution in which incidence increases and peaks between the ages of 20 to 29 years, decreases between the ages of 30 to 54 years, and then increases again after the age of 55 years.[32,33] Little is known about risk factors for specific subtypes of HL. Furthermore, the deaths were classified according to the International Classification of Diseases, Eighth Revision, in the database, which does not allow investigation of specific subtypes of HL.

Overall, the absence of increased risks in other occupational cohorts and the lack of a plausible biological mechanism for chemical exposures in the etiology of HL detract from a causal explanation for the observed association in this study. The small numbers of HL deaths increase the likelihood that random error contributed to the observed patterns.

For ML, initial Cox proportional hazards analyses suggested an association of similar magnitude for both categories of peak exposure (ie, 2 ppm or more to less than 4 ppm and 4 ppm or more in separate analyses compared with the same "no peaks" referent). Nevertheless, among the MLs, a stronger association with peak exposure was seen for CML than for AML. The clear lack of an association with cumulative exposure, the default dose metric in most epidemiologic studies, for both CML and AML further weakens arguments for causal attribution. Moreover, and in contrast to HL, there is no indication of an excess mortality due to AML in this cohort, even after assuming that all 21 "unspecified" acute leukemias were AMLs. Our SMR analysis confirmed a deficit of MLs of more than 30% among the unexposed, but only a small deficit of AML among the unexposed. In contrast, 13 deaths from CML were observed among the exposed group (compared with approximately 13 expected), and 30 AMLs were observed among the exposed group (compared with approximately 38 expected) (Table 2). It is possible that there may be some underlying differences between the nonexposed and exposed subcohorts, such that a deficit of MLs among the nonexposed gave rise to an apparent association in analysis using an internal referent. Many of the LHM deaths occurred among the short-term workers, who might have had the least opportunity to accumulate exposure, and were half as likely to have worked in jobs classified as having peak exposures. Conversely, workers who remain unexposed over their entire duration of employment were more likely to have worked as technicians or white-collar employees rather than as production workers or laborers, and differences in results between the two groups may reflect socioeconomic differences. Other studies have shown increased risks of AML of similar magnitude among professionals, including groups unexposed to formaldehyde or any chemicals, such as priests (Standardized Incidence Ratio = 1.75; 95% CI, 1.20 to 2.47).[34]

Other cohorts of formaldehyde-exposed workers have not demonstrated notable associations with ML. In the original analyses of the British cohort, Coggon et al[29] reported no excess of leukemia deaths overall (SMR = 0.91; 95% CI, 0.47 to 1.59) or among the subcohort with high formaldehyde exposure (SMR = 0.71; 95% CI, 0.31 to 1.39) estimated from limited exposure monitoring data and worker reports of irritant symptoms; however, results for ML or its subtypes AML and CML were not provided. The most recent update of that cohort[30] also reported no excess of leukemia deaths overall (SMR = 1.02; 95% CI, 0.77 to 1.33) or among the high formaldehyde exposure subcohort (SMR = 0.82; 95% CI, 0.44 to 1.41). Analyses of ML deaths were similar for the total cohort (SMR = 1.2; 95% CI, 0.84 to 1.66) and for the high formaldehyde exposure subcohort (SMR = 0.93; 95% CI, 0.40 to 1.82); however, results for AML deaths were not presented. No associations with any of the other LHM were observed among the total cohort or among the high formaldehyde exposure group. The US NIOSH garment workers cohort had suggested an association between formaldehyde and leukemia; however, the authors recently reported that the extended follow-up of this cohort "did not strengthen previously observed associations."[31] The interpretation of results of extended follow-up of all of these cohorts becomes more complicated, however, as background rates of AML increase 30-fold from aged 50 to 59 years to 80 years and older,[33] and these are less likely to be related to workplace exposures from decades earlier.

Leukemias have shorter latencies than solid tumors, which often manifest 20 or more years after exposure. Studies of atomic bomb survivors in Japan found that AML incidence peaks between 5 and 7 years after radiation exposure and declines over time.[35,36] Deschler and Lubbert[37] reported that the incidence of AML following chemotherapy peaks 5 to 10 years after treatment. The American Cancer Society reported that AML following treatment with topoisomerase inhibitors occurs within 2 to 3 years.[38] In addition, AML occurring in older ages may be coincidental and unrelated to any relevant occupational exposure that occurred in the distant past; yet these older AML cases could inflate the apparent latency.[39–42] Reasonable estimates for the maximum latency for acute leukemia associated with intense occupational exposure to benzene seem to be in the range of 5 to 10 or possibly 15 years.[26,27] Applying these latencies to the NCI industrial workers cohort, there is no clear evidence of an association with any exposure to formaldehyde, including peak exposure either as originally defined or as we redefined it.

Evaluation of other LHMs in the NCI cohort demonstrated no associations with cumulative or peak formaldehyde exposure metrics, consistent with other cohorts.

Reliance on mortality data for LHM may miss incident cases. This is especially true for HL for which the 5-year relative survival increased from 72% for the period 1979 to 1980 to approximately 88% for the period 2003 to 2009.[33] In contrast, the 5-year relative survival for AML increased from approximately 8% for the years 1978 to 1980 to approximately 25% during 2003 to 2009,[33] although 5-year relative survival is lower for individuals diagnosed at the age of 65 years and older.[33] Nevertheless, most AML deaths occurred more than 20 years after the last possible peak formaldehyde exposure, suggesting that marginally improved survival rates unlikely masked underlying true associations.

A further consideration for interpreting our findings is that biological mechanisms for the induction of leukemia by exogenous formaldehyde have not been established. Recent experimental studies have applied sensitive methods to distinguish endogenous formaldehyde concentrations in tissue from concentrations that result from exogenous formaldehyde exposure and have shown that formaldehyde present in protein adducts detected in the bone marrow derives exclusively from endogenous formation.[6,7] Formaldehyde does not form DNA:protein crosslinks[43,44] or DNA adducts[6] in bone marrow. The mounting mechanistic evidence is consistent with the body of epidemiological evidence—including these additional analyses of the NCI formaldehyde workers cohort—that occupational formaldehyde exposure does not increase risk of AML.

CONCLUSIONS

We replicated the associations of cumulative and peak formaldehyde exposures with HL previously reported from this cohort. Causal interpretations for the replicated associations with HL and the unanticipated association with CML are uncertain due to the absence of corroborative evidence from other epidemiologic studies of formaldehyde-exposed cohorts. Furthermore, the absence of established pathogenesis mechanisms for HL and CML raises doubt as to whether these observed associations are causal.

No other clear associations for peak or cumulative formaldehyde exposures were observed in this cohort for any of the specific LHM, including AML. Although our re-analysis using redefined "peak" exposure detected associations similar to those previously reported with the combined MLs, our new analyses of AML and CML mortality separately suggest that the observed patterns with

peak exposure were confined to CML. Furthermore, when taking into account the timing of peak exposure, no increased risk for AML is seen, as only one AML death occurred within 15 years of first, or even last, peak exposure. Sensitivity analyses assuming all the "unspecified" acute leukemia deaths were AMLs did not change these findings.

Our re-analysis of the data from the NCI cohort study of workers in the formaldehyde industries provides no support for the hypothesis that formaldehyde causes AML, the LHM of greatest prior concern.

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